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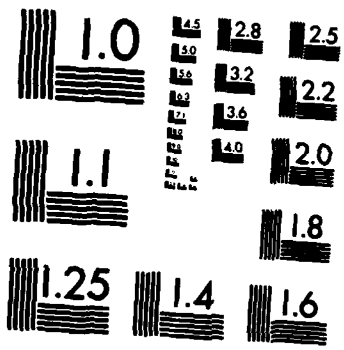

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COMPARATIVE STUDIES OF THE SHORT-TERM TOXICITY OF THE HYDRAULIC FLUIDS MIL-H-19457C, MIL-H-19457B, AND MIL-H-22072B

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NOTICES

TECHNICAL REVIEW AND APPROVAL

AAMRL-TR-86-030

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

BRUCE O. STUART, PhD
Director Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

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			MIL-H-22072B	Ethylene Glycol	

19. ABSTRACT *(Continue on reverse if necessary and identify by block number)*

Acute toxicity studies including eye and skin irritation, skin sensitization, single dose oral, single dose intraperitoneal, 4-hour aerosol inhalation, single dose dermal, and 21-day repeated aerosol inhalation were conducted with the hydraulic fluids MIL-H-19457C, MIL-H-19457B and MIL-H-22072B. The first two hydraulic fluids are triarylphosphate ester-based materials, while the third is glycol based. MIL-H-19457C and MIL-H-19457B were also tested for neurotoxic potential with a repeated dose screening assay in chickens. None of the three hydraulic fluids produced eye or skin irritation in rabbits. Guinea pig sensitization tests were also negative for all three fluids. Single oral doses of 5 mL/kg of any of the materials failed to produce mortality in male or female Sprague-Dawley rats. Diarrhea and weight loss were seen in the rats given either MIL-H-19457C or MIL-H-19457B. These effects were not seen in the rats dosed with MIL-H-22072B. Single dose intraperitoneal LD_{50} values were also determined.

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Block 11 (continued)

Toxicity of the Hydraulic Fluids MIL-H-19457C, MIL-H-19457B, and MIL-H-22072B

PREFACE

This document constitutes the final report on the Comparative Studies of the Acute Toxicity of the Hydraulic Fluids MIL-H-19457C, MIL-H-19457B, and MIL-H-22072B. The research covered a period from December 1981 through May 1985 and was performed under Contract No. F33615-80-C-0512. M.K. Pinkerton served as technical contract monitor for the Harry G. Armstrong Aerospace Medical Research Laboratory.

The work was sponsored by the U.S. Navy under the direction of LCDR M.J. Cowan, Jr., MSC, USN, Ret.; and Capt. D.E. Uddin, MSC, USN; and identified as Work Unit Number MF-5854001.006.

J.D. MacEwen, Ph.D., served as the Laboratory Director for the THRU of the University of California, Irvine, and as co-principal investigator with T.T. Crocker, M.D., Department of Community and Environmental Medicine. Acknowledgment is made to D.E. Uddin, C.D. Flemming, and J.L. Monroe for their significant contributions and assistance in the preparation of this report.

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INTRODUCTION

Hydraulic fluids of many types are utilized by the U.S. Navy. Engineering specifications impose chemical and physical criteria which cannot always be satisfied by toxicologically inert materials.

Exposure to hydraulic fluids in the naval environment occurs from inhalation of vapors and aerosols and from direct skin contact. Aerosols are often generated from leaks in high pressure systems. Skin contact results from material handling, systems maintenance, and damage control operations.

Of the many types of hydraulic fluids used by the Navy, the fire resistant, phosphate ester-based type described by Military Specification H-19457 has caused the greatest health concern because of the known neurotoxicity of some members of this class of chemicals. Triorthocresyl phosphate (TOCP) is the best known and most neurotoxic example of the phosphate esters present in hydraulic fluids. TOCP occurred as a contaminant in a tricresyl phosphate hydraulic fluid used in earlier naval operations. To reduce potential health hazards, this material was replaced with a less neurotoxic material, tri-(tertiary butylphenyl) phosphate. New technology will allow for future use of water-glycol-based hydraulic fluids. Presumably, these water-glycol fluids will be less toxic than the phosphate ester-based materials.

Changing hydraulic fluids would necessitate major equipment modifications at great expense, and studies involving evaluation of the toxic effects of the various hydraulic fluids are necessary to properly compare the health risks associated with the various hydraulic fluids.

The Naval Medical Research Institute, Toxicology Detachment (NMRI/TD), requested that the THRU conduct a series of acute toxicity studies with three hydraulic fluids. Two of the fluids, MIL-H-19457C and MIL-H-19457B, are phosphate ester based, while the third, MIL-H-22072B, is water-glycol based.

The most significant routes of industrial exposure to the hydraulic fluids are expected to be dermal, as a result of spills or leaks, and aerosol inhalation, from pressurized system leaks. The list of acute studies conducted, shown below, reflects these routes of exposure.

1. Skin Irritation
2. Eye Irritation
3. Skin Sensitization
4. Acute Oral Toxicity
5. Acute Intraperitoneal Toxicity
6. Acute Inhalation Toxicity
7. Acute Dermal Toxicity
8. 21-Day Repeated Dose Inhalation Toxicity

Since MIL-H-19457C and MIL-H-19457B are phosphate ester-based hydraulic fluids, a screening test was also conducted to determine if delayed neurotoxic effects would result from exposure of adult chickens to these two hydraulic fluids. A vehicle control (corn oil) and a TOCP positive control were tested concurrently with samples of MIL-H-19457B and MIL-H-19457C. The final determination of injurious effect was based on a comparison of the hydraulic-fluid-dosed chickens with the TOCP-dosed control chickens.

MATERIALS AND METHODS

ANIMALS

For the acute studies, male and female New Zealand White rabbits weighing approximately five pounds were purchased from Willoughby's Rabbitry (Sabina, OH) and Sweetwater Farm, Incorporated (Hillsboro, OH). Male and female Sprague-Dawley rats (150-250 g) were purchased from Charles River Breeding Laboratories (Wilmington, MA). Male Hartley-derived, albino guinea pigs (300-500 g) were purchased from Murphy's Breedings Labs. (Plainfield, IN). Rabbits were individually maintained in stainless steel wire mesh cages; rats and guinea pigs were housed in polycarbonate cages with wood chip bedding. Food and water were available *ad libitum*.

For the 21-day inhalation studies, male and female Fischer 344 rats (8-10 weeks of age) and male Golden Syrian hamsters (8-10 weeks of age) were purchased from Charles River Breeding Laboratories (Wilmington, MA). Male rabbits (3-4 kg) used in the MIC-H-19457C and MIL-H-19457B exposures were purchased from Ancare Corporation (Manhasset, NY). Female rabbits (3-4 kg) used in these same studies were purchased from Dutchland Labs, Inc. (Denver, PA). Male and female rabbits (3-4 kg) used in the MIL-H-22072B exposures were purchased from Plummers Rabbit Ranch (Peebles, OH). All animals were housed in stainless steel cages with wire mesh floors during the exposure. Water was available *ad libitum*. Food was withheld during the 6-hour inhalation exposure period, but was available *ad libitum* during nonexposure hours.

Leghorn hens 12 to 14 months of age and weighing between 1.2 and 1.8 kg were purchased from Carey Farms (LaRue, OH). The debeaked hens were group-housed in 3- by 6-ft cages to allow for freedom of movement. Food and water were available *ad libitum*.

TEST MATERIALS

Samples of the three hydraulic fluids were supplied by the NMRI/TD, Wright-Patterson Air Force Base, OH. Lot number identification and physical and chemical properties of the hydraulic fluids follow:

Mil. Spec. No:	MIL-H-19457C
NMRI/TD Sample Number:	1257-2
Lot Number:	0820-E-1-1
Specific Gravity (60°/60°F):	1.150
Boiling Point (°F):	735
Odor:	Very slight
Chemical Composition:	tri-(tertiary butylphenyl)phosphate

Mil Spec. No.:	MIL-H-19457B
NMRI/TD Sample Number:	1257-3

No other chemical property data available.

Mil. Spec. No:	MIL-H-22072B
NMRI/TD Sample Number:	1257-1
Lot Number:	B9A
Specific Gravity:	1.08
Boiling Point (°F):	220
Odor:	Slightly ammoniacal
Chemical Composition:	33-35% ethylene glycol, 43.5-51.5% water, and 14-24% polyglycol

SKIN IRRITATION

Six rabbits were clipped of all possible hair on the back and flanks 24 hours before exposure to allow for recovery of the skin from any abrasion resulting from the clipping. Two areas on the back,

one on each side, were designated as patch-test areas. One area was abraded by making minor incisions through the stratum corneum. These abrasions were not sufficiently deep to disturb the derma or to produce bleeding.

The hydraulic fluid was applied as 0.5 mL to the designated patch-test area and was covered by a 1-inch square of surgical gauze two single layers thick. The gauze patches were held in place with strips of Elastoplast® tape. The entire area was covered with rubber dental dam strip and secured with more Elastoplast® tape. The patches remained in place for 24 hours. During that time, the rabbits wore leather restraining collars to prevent disturbance of the patch area while allowing freedom of movement and access to food and water.

After 24 hours, the wrap and patches were carefully removed, and the test areas were examined for irritation using the Draize (1959) table as a reference standard. Readings were also made at 72 hours (48 hours after the first reading). The total score of both readings for all six rabbits was divided by 24 to yield a primary irritation score.

EYE IRRITATION

A 0.1-mL sample of hydraulic fluid was applied to one eye of each of six to nine albino rabbits. The opposite eye was untreated and served as a control. When nine rabbits were used, the treated eyes of three rabbits were flushed with lukewarm water approximately 30 seconds after instillation of the fluid. Examinations for gross signs of eye irritation were made at scheduled observation periods following application. Scoring of the irritative effects was according to the method of Draize (1959).

MODIFIED MAGUIRE SKIN SENSITIZATION TEST

Ten male albino guinea pigs were used to test the skin sensitization potential of each hydraulic fluid. An area on the back of each animal directly above the forelegs was clipped with electric clippers and the fur chemically removed with a commercial depilatory on the morning of the first insult exposure as recommended by Maguire (1973). Test solutions, 0.1 mL at each application, were applied to this area on a 0.5-inch cotton gauze square, covered with dental dam, and held in place with adhesive tape. The first insult patch was allowed to remain in place for two days, then removed, and a second application of 0.1 mL was made. Two days later, this patch was removed, a total of 0.2 mL of Freund's adjuvant per animal was injected intradermally, using two or three points adjacent to the insult site, and then a new patch containing 0.1 mL of the test material was applied. Three days after the third application, a fresh patch containing 0.1 mL of the material was applied. The last patch was removed two days later, and the animals were allowed to rest for two weeks. Each time the insult patches were removed, the condition of the skin at the application site was graded and recorded. When the last patch was removed, the toes on the hind feet of the guinea pigs were taped to prevent them from scratching the irritated area.

After the two-week rest period, the right flanks of the same animals were clipped and challenged with the test solution. The challenge application was not occluded. The skin response at these sites was recorded at 24 and 48 hours after application according to the evaluation method of Draize (1959), shown in Table 1. Any animal eliciting a score of 2 or more at the test solution challenge site would be rated as a positive responder.

TABLE 1. GRADING OF SKIN REACTIONS IN THE MODIFIED MAGUIRE SKIN SENSITIZATION TEST

Erythema	Edema
0 – None	0 – None
1 – Very slight pink	1 – Very slight
2 – Slight pink	2 – Slight
3 – Moderate red	3 – Moderate
4 – Very red	4 – Marked

ACUTE ORAL AND INTRAPERITONEAL TOXICITY

Syringes equipped with special oral dosing needles were used to administer the hydraulic fluids to male and female Sprague-Dawley rats. Rats used for oral dosing studies were fasted 12 hours before dosing. Solutions of the hydraulic fluids were prepared in distilled water (MIL-H-22072-B) or corn oil (MIL-H-19457C and MIL-H-19457B). A single dose at a volume equivalent to 1 percent of the animal's body weight was given.

Testing was initiated by dosing five rats of each sex at a concentration of 5 mL/kg body weight. This concentration was used as an upper limit cutoff level. If no toxicity was evident during a 14-day observation period, no further testing was conducted. If mortality was produced, testing continued with 10 animals of each sex per dose level. Mortality was recorded for 14 days after dosing. A 14-day LD_{50} with 95 percent confidence limits was calculated using the probit analysis method of Finney (1971).

ACUTE INHALATION TOXICITY

Male and female Sprague-Dawley rats weighing between 200-300 g and 150-250 g, respectively, were used for determination of the acute 4-hour LC_{50}.

One three-port Collison® nebulizer operated at 30 psi was used for generation of the aerosol. A three-neck, round-bottom flask was used to contain the hydraulic fluid sample pool. A single sample pool was used without replacement during the four-hour exposures that were conducted in a 60-L plastic chamber. Airflow through the chamber was maintained at about 10 L/min. Aerosol was sampled using three midget impingers connected in series. Isopropanol was used as a diluent and sampling solution for MIL-H-19457C and MIL-H-19457B. Distilled water was used for MIL-H-22072B. Total sample air volume was 1 L. Particle sizing was accomplished with an Aries seven-stage impactor. Respective stages were pooled and eluted in isopropanol or water for analysis (three samples/hour for MIL-H-19457C; four samples every two hours for MIL-H-19457B and MIL-H-22072B. A McPherson UV/Vis spectrophotometer was used for analysis of MIL-H-19457C (260 nm, 1-cm cell) and MIL-H-19457B (265 nm, 1-cm cell). A Waters liquid chromatogram M-6000A with an R401 differential refractometer was used for MIL-H-22072B analysis.

Testing was initiated by exposing five rats of each sex to an upper limit cutoff concentration of 5 mg/L. If no toxicity was evident during a 14-day observation period, no further testing was conducted.

ACUTE DERMAL TOXICITY

Male and female New Zealand white rabbits were used for determining the acute dermal LD_{50}. All rabbits were clipped as closely as possible with an Oster® clipper having surgical blades and vacuum attachment. The backs of the rabbits and the sides about halfway to the stomach area were clipped.

The hydraulic fluid was applied in equal amounts to both sides of the back and remained in contact with the skin for 24 hours. The dose was kept in place by applying 4- by 4-inch, 8-ply gauze patches over the compound on each side of the back. Latex rubber dental dam was then applied over the entire clipped area, and Elastoplast® tape was used to wrap the entire midsection, keeping the dose in place. Specially designed restraining harnesses were fitted to each rabbit at the time of dosing and kept in place during the entire dosing period. These harnesses prevented excessive movement of the rabbits and chewing on the taped area while allowing the rabbits access to food and water. Upon removal of the wrapping, the skin of the rabbit was wiped (not washed) to remove excess test material.

Testing was initiated by dosing five rabbits of each sex at an upper limit cutoff concentration of 2 mL/kg body weight. If no toxicity was evident during the 14-day observation period, no further testing was conducted.

OBSERVATION (ORAL, INTRAPERITONEAL, INHALATION, AND DERMAL TESTS)

Animals from the acute dermal, oral, intraperitoneal, and inhalation tests were observed frequently during the day of dosing and twice daily during the 14-day holding period. Visible signs of toxicity were recorded. Body weights of all animals were obtained at the time of dosing and periodically during the 14-day holding period.

All animals, whether dying during the test or sacrificed at the conclusion of the 14-day observation period, were necropsied, and any gross changes were recorded following death. Histopathologic examination was performed on any abnormal tissue observed in animals from the acute oral and intraperitoneal toxicity tests. Lung, trachea, liver, kidney, and abnormal tissues were examined for histopathologic alterations from animals exposed via inhalation.

21-DAY REPEATED DOSE INHALATION STUDIES

NMRI/TD provided information of industrial hygiene surveys aboard ship which indicated maximum aerosol concentrations of 25 mg/m^3 (8 mg/m^3 average). These surveys were conducted for the glycol-based materials and were thought to be similar to levels of aerosol produced when triarylphosphate fluids were used in similar shipboard operations. Based on these data, a level of 25 mg/m^3 was chosen as the low-level exposure and 250 mg/m^3 for the high-level exposure.

The experimental regimens for MIL-H-19457C, MIL-H-19457B, and MIL-H-22072B were identical. Groups consisted of 10 male and 10 female Fischer 344 rats, 10 male Golden Syrian hamsters, and 4 male and 4 female New Zealand white rabbits. Exposure to the hydraulic fluid aerosol was conducted on a 6-hour/day, 5-day/week basis. Exposures were not conducted on weekends. All animals were killed for tissue collection and evaluation on day 21 of the study. Fifteen exposures were conducted during the 21-day period in the 25-m^3 Thomas Dome inhalation chambers.

The MIL-H-19457C and MIL-H-19457B studies each had chamber control groups of 10 male and 10 female rats and 10 male hamsters. Because of limited cage space in the chamber, it was not

possible to maintain separate rabbit control groups for each test material. Therefore, a control exposed group of 4 male and 4 female rabbits was used for both studies. The MIL-H-19457C and MIL-H-19457B studies were initiated one week apart. Because of technical difficulties with the generation and chemical analysis system for the MIL-H-22072B study, it was necessary to conduct these exposures at a later date. A separate chamber control group of rats, hamsters, and rabbits was maintained for this study

Both MIL-H-19457C and MIL-H-19457B aerosols were generated with multi-jet, Collison® nebulizers mounted in 250-mL distillation flasks. Aerosol output of the generators was diluted with the airstream in the chamber input air line. Chamber concentration was controlled with adjustments to nebulizer back pressure and/or chamber airflow. High-level exposure concentrations were determined by analysis of filter samples eluted with reagent-grade isopropyl alcohol and read on a GCA/McPherson UV/Vis spectrophotometer at a wavelength of 260 nm. Filter samples were obtained by drawing chamber air through Gelman Metricel filters (0.45 µm) mounted in open-faced holders. The sampling system was flow-calibrated daily with a wet test meter at a rate of 5.0 L/min.

A second analysis method was employed in the low-level exposures using a TSI aerosol photometer, with instrumental output standardized on the basis of the same filter sample method. The aerosol photometer has a continuous unattended monitoring capability and operates on the principle that the intensity of scattered light is proportional to particle concentration. A photodiode measures the intensity of scattered light at a 90° angle from a focused incandescent tungsten source, which may be read as percentage of full-scale displacement on a chart recorder and translated into a concentration value by an experimentally determined mass concentration coefficient. The system was designed to alternate reading of chamber aerosol and chamber input air, the latter providing a baseline value. A series of valves were synchronized to operate in a staggered on-off sequence. The resulting sampling cycle was 20 min in duration, a 4-min chamber sample followed by a 16-min air purge. High and low concentration alarm points were established to give warning of system failure.

MIL-H-22072B is a water, ethylene glycol, and polyglycol mixture. The former two components almost completely volatilize during aerosol generation while the nonvolatile polyglycols remain in the liquid phase. Since "real life" aerosols of MIL-H-22072B are produced by leaks in high pressure hydraulic systems, it is reasonable to assume that the actual use situation presents an environment of both ethylene glycol vapor and polyglycol aerosol. It was therefore decided that the experimental atmosphere would contain both vapor and aerosol components of MIL-H-22072B.

Before exposure was initiated, it was established that the composition of MIL-H-22072B was 37.5 percent ethylene glycol and 15.0 percent polyglycol. Consequently, the 25-mg/m^3 MIL-H-22072B exposure level translated to 9.37 mg/m^3 ethylene glycol vapor and 3.75 mg/m^3 polyglycol aerosol. Similarly, the high-level exposure at 250 mg/m^3 became 93.7 mg/m^3 ethylene glycol vapor and 37.5 mg/m^3 polyglycol aerosol.

Aerosol generation systems for both the high- and low-level MIL-H-22072B exposures employed multi-jet, Collison® nebulizers mounted in distillation flasks with concentration controlled by adjusting nebulizer back pressure and/or chamber airflow.

The metal surfaces of the chambers and introduction lines, as well as water within the chamber, quickly adsorbed or absorbed the ethylene glycol vapor generated from the nebulizer We found that once these surfaces were passivated, the ethylene glycol vapor concentration remained relatively stable. Therefore, it was necessary to add pure ethylene glycol to the high-level chamber at the start-up of each 6-hour exposure to bring the ethylene glycol concentration quickly to the desired level. The amount used was decreased once the desired concentration was reached. The

total ethylene glycol used daily varied, but averaged about 40 mL. The ethylene glycol was pumped with a polystaltic pump into two resistance-coil-wrapped, glass towers. The output airflow temperature was maintained at 70 ± 3°C. Stainless steel lines from the tower output to the contaminant vents were wrapped with a heating tape.

Ethylene glycol vapors were analyzed by pulling exposure chamber air samples (filtered to remove aerosol) through a Miran 1A infrared spectrophotometer. Aerosol concentration was determined with a TSI aerosol photometer in a manner similar to that described previously for the MIL-H-19457C and MIL-H-19457B exposures.

Particle size distribution analysis was performed once daily for all exposures using a high-volume, eight-stage, cascade impactor. The size-segregated particles were impacted on preweighed aluminum collection discs and analyzed gravimetrically. Mass per stage was converted to cumulative percentage mass, then probits. Log of particle diameter versus probit plot was generated by a weighted linear regression calculation, from which mass-median diameter and geometric standard deviation were obtained. Calculations were performed by a software program developed for the Hewlett-Packard 3388A computer/integrator.

All animals were closely observed during the 21-day period. Individual body weights were measured twice weekly. Blood samples were taken from all rats and rabbits at exposure termination for the tests listed in Table 2. All animals were fasted 18 hours prior to collection. EDTA was used as the anticoagulant for the hematology samples, and the chemistry tests were performed on serum collected from clotted samples.

TABLE 2. CLINICAL HEMATOLOGY AND CHEMISTRY TESTS PERFORMED ON RATS AND RABBITS EXPOSED TO HYDRAULIC FLUID AEROSOLS

Hematology	Chemistry[a]
Hematocrit	Calcium
Hemoglobin	Total Protein
RBC	Albumin
WBC	Alkaline Phosphatase
Differentials	SGOT
Mean Corpuscular Volume (MCV)	Creatinine
Mean Corpuscular Hemoglobin (MCH)	BUN
Mean Corpuscular Hemoglobin Concentration (MCHC)	

[a]Samples were sent to NMRI/TD for analysis.

Whole-body, brain, liver, kidney, spleen, and heart weights were measured on all rats sacrificed at exposure termination. At the conclusion of the exposure phase of the study, all animals were killed for gross examination and tissue collection for histopathologic examination (Table 3).

Data from routine animal weighing, hematology, blood chemistry, and organ weighing were analyzed for statistical significance using a Student t-test.

TABLE 3. TISSUES COLLECTED FOR HISTOPATHOLOGIC EXAMINATION OF ANIMALS EXPOSED TO HYDRAULIC FLUID AEROSOLS

Gross lesions	Heart
Tissue masses or suspect tumors and regional lymph nodes	Liver
	Spleen
Larynx	Kidneys
Trachea	Brain
Lung and bronchi	Sciatic nerve

NEUROTOXICITY

The hydraulic fluids, as well as the positive control TOCP, were administered to unfasted hens as solutions in corn oil. Gastric intubation was accomplished employing a syringe fitted with a 6-inch infant catheter. The injection volume for the hens was calculated in milliliters per kilogram, which resulted in the average chicken receiving a volume of 1.5 mL. The chickens were weighed individually to determine the proper dosage volume.

The following regimen of dosing was performed on five consecutive days:

MIL-H-19457B – Groups of four hens each treated with the following doses: 240, 300, 360, and 420 mg/kg/day.

MIL-H-19457C – Groups of four hens each treated with the following doses: 240, 300, 360, and 420 mg/kg/day.

TOCP – Groups of four hens each treated with the following doses: 60, 75, and 90 mg/kg/day.

Corn Oil – Twelve hens given 1 mL/kg/day.

Grading by three observers began seven days after the first dose and continued three times/week (Monday, Wednesday, and Friday), until 30 days after the initial dose. The following point score system was used.

Symptom Free	0 Points
Doubtful or Minor Symptoms	2 Points
Positive Paralytic Symptoms	8 Points
Advanced Paralytic Symptoms	12 Points
Death	16 Points

During observation and grading, the chickens were removed from their enclosures and placed on a rubber mat to provide sure footing. Symptoms noted on the 21st day after the first dose were used for calculating the TOCP equivalent.

The calculation was completed as follows:

$$TOCP\ Equivalent(\%) = \frac{mg/kg\ TOCP}{mg/kg\ Test\ Material} \times \frac{Total\ Points\ for\ Test\ Material \times 100}{Total\ Points\ for\ TOCP}$$

Following the final observation day, nerve tissue was sampled from four chickens from each of the following groups: Vehicle Control, TOCP (two 75-, two 90-mg/kg/day chickens), MIL-H-19457B (240 mg/kg/day), and MIL-H-19457C (420 mg/kg/day). Nerve tissue was taken from the cervical, thoracic, and lumbar regions of the spinal cord, as well as a sample of peripheral nerve (sciatic).

RESULTS

PRIMARY SKIN IRRITATION

None of the three hydraulic fluids, when applied undiluted to intact and abraded rabbit skin, produced a primary irritation response, and therefore, they are not considered primary skin irritants for rabbits.

PRIMARY EYE IRRITATION

None of the three hydraulic fluids caused any ocular irritation in the rabbits. No differences were noticed when comparing the exposed eyes, washed or unwashed, with the respective control eyes at the scheduled observation periods.

SKIN SENSITIZATION

None of the hydraulic fluid samples tested by the modified Maguire method caused a dermal reaction in guinea pigs. The responses to the challenge applications of the hydraulic fluids to 10 guinea pigs following a two-week incubation period were negative in all three cases.

ORAL TOXICITY

Doses of 5 mL/kg of any of the three hydraulic fluids failed to produce mortality during the 14-day observation period. Diarrhea, first noted about 6 hours after dosing and lasting one or two days, was evident in rats given the MIL-H-19457C or MIL-H-19457B. The diarrhea coincided with a weight loss in the majority of rats dosed with either of these two triarylphosphate materials (Table 4). When the diarrhea subsided, normal and steady weight gains were evident. Rats dosed with MIL-H-22072B exhibited neither diarrhea nor weight loss.

Gross necropsy failed to reveal any significant exposure-related lesions. Microscopic examination of a limited number of rats indicated mild pulmonary changes, which were considered to be unrelated to oral exposure.

Because no mortality resulted at a concentration of 5 mL/kg, no further oral toxicity studies were conducted.

INTRAPERITONEAL TOXICITY

Rats injected with MIL-H-19457C or MIL-H-19457B were mildly lethargic for 5 to 10 hours, and a clear oily discharge was noticed around the anus of the rats. One male rat injected with MIL-H-19457C died within 24 hours of dosing. All other male and female rats given MIL-H-19457C or MIL-H-19457B survived the 14-day observation period, and no overt signs of toxicity were noted subsequent to the initial lethargy. Rats injected with MIL-H-22072B became severely lethargic. Signs of toxicity included slow, labored respiration, sprawling of the hind limbs, and extremely depressed

fighting and placement reflex. These symptoms lasted from 24 to 48 hours. Despite the initial depressed state, all rats survived the 14-day observation period.

TABLE 4. BODY WEIGHTS (g)[a] OF RATS GIVEN A SINGLE ORAL DOSE (5 mL/kg) OF HYDRAULIC FLUID

Day	MIL-H-19457C	MIL-H-19457B	MIL-H-22072B
		Males	
0	272 ± 16	252 ± 22	275 ± 27
1	268 ± 11	248 ± 20	289 ± 28
2	278 ± 17	246 ± 12	298 ± 17
4	294 ± 23	258 ± 13	310 ± 28
7	309 ± 25	282 ± 17	333 ± 31
14	342 ± 30	320 ± 18	369 ± 31
		Females	
0	174 ± 8	165 ± 7	172 ± 6
1	167 ± 11	168 ± 10	186 ± 11
2	182 ± 9	168 ± 13	188 ± 11
4	191 ± 7	174 ± 12	194 ± 11
7	197 ± 10	192 ± 7	199 ± 11
14	213 ± 11	202 ± 5	212 ± 7

[a]Mean ± SD, N = 5 rats/group.

Body weights are shown in Table 5. Weight loss was seen one or two days following injection of any of the hydraulic fluids. By one week, the rats began to gain weight and subsequently showed normal weight gain.

Since no rats died at intraperitoneal doses of 5 mL/kg of MIL-H-19457B or MIL-H-22072B, no further intraperitoneal tests were conducted with these materials. The death of the one male rat given 5 mL/kg of MIL-H-19457C prompted further intraperitoneal tests. Results of the tests and calculated LD_{50} values are shown in Table 6. The LD_{50} values for male and female rats are very comparable, and both are greater than 10 mL/kg, indicating a low toxicity hazard. No deaths occurred in the group of 10 male or female rats given 5.0 mL/kg. Injections at doses greater than 14.3 mL/kg were not attempted.

Body weights are shown in Figures 1 and 2 for male and female rats, respectively. All of the male rats given MIL-H-19457C intraperitoneally experienced an initial weight loss lasting one to two days. Male rats dosed at the higher concentrations had a more prolonged weight loss lasting up to four days. A dose-related effect on weight recovery was evident in the male rats. In fact, the male group given 14.3 mL/kg never did attain its preexposure body weight during the 14-day observation period.

TABLE 5. BODY WEIGHTS (g)[a] OF RATS GIVEN A SINGLE INTRAPERITONEAL INJECTION (5 mL/kg) OF HYDRAULIC FLUID

Day	MIL-H-19457C	MIL-H-19457B	MIL-H-22072B
		Males	
0	265 ± 19	254 ± 10	256 ± 7
1	242 ± 16	240 ± 15	247 ± 10
2	237 ± 20[b]	241 ± 16	239 ± 5
4	250 ± 20[b]	249 ± 18	244 ± 7
7	273 ± 21[b]	264 ± 22	261 ± 8
10	301 ± 23[b]	283 ± 20	278 ± 11
14	321 ± 22[b]	305 ± 20	300 ± 14
		Females	
0	200 ± 20	190 ± 13	194 ± 7
1	182 ± 18	181 ± 14	183 ± 9
2	180 ± 15	180 ± 14	190 ± 10
4	190 ± 18	177 ± 14	195 ± 11
7	205 ± 25	188 ± 13	203 ± 8
10	213 ± 28	201 ± 13	212 ± 11
14	221 ± 21	205 ± 17	221 ± 12

[a]Mean ± SD, N = 5 rats/group.

[b]N = 4.

TABLE 6. ACUTE INTRAPERITONEAL TOXICITY OF MIL-H-19457C

Dose (mL/kg)	Male Rats	Female Rats
Corn oil[a]	0/10[b]	0/10
5.0	0/10	0/10
6.5	0/10	0/10
8.5	2/10	4/10
11.0	6/10	3/10
14.3	7/10	6/10
LD_{50}	11.2 mL/kg	12.4 mL/kg
95 percent confidence limits	9.7 to 13.7	10.3 to 19.4

[a]Not used in LD_{50}.

[b]Number dead/Number dosed.

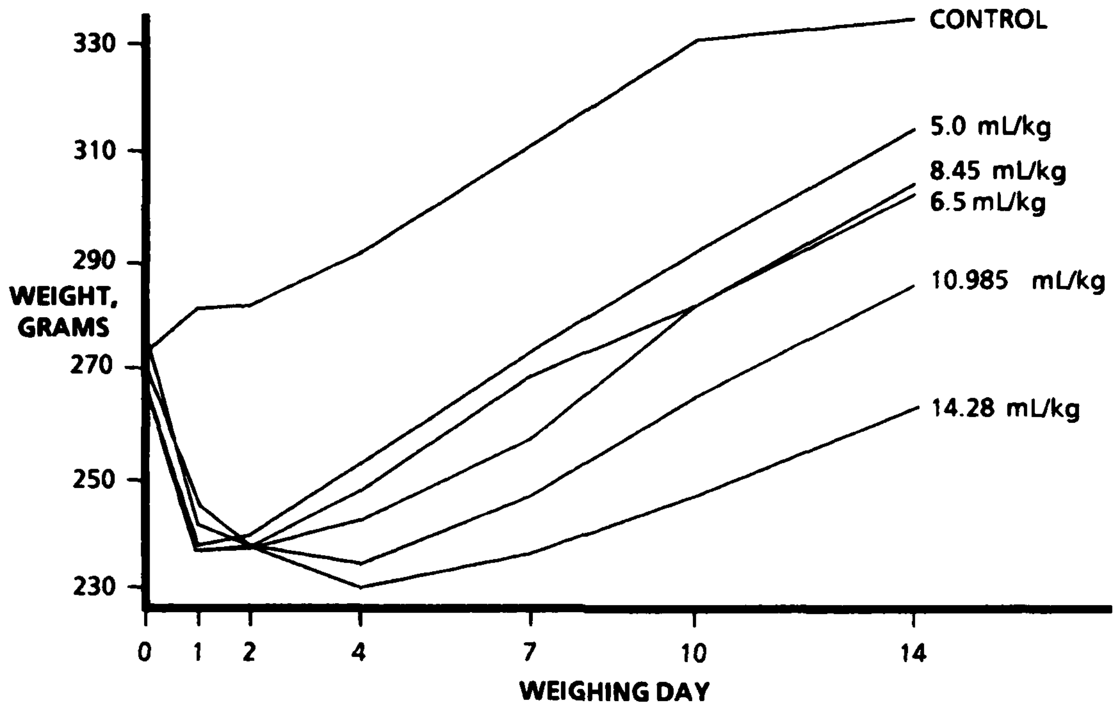

Figure 1. Body weights of male rats given intraperitoneal injections of MIL-H-19457C.

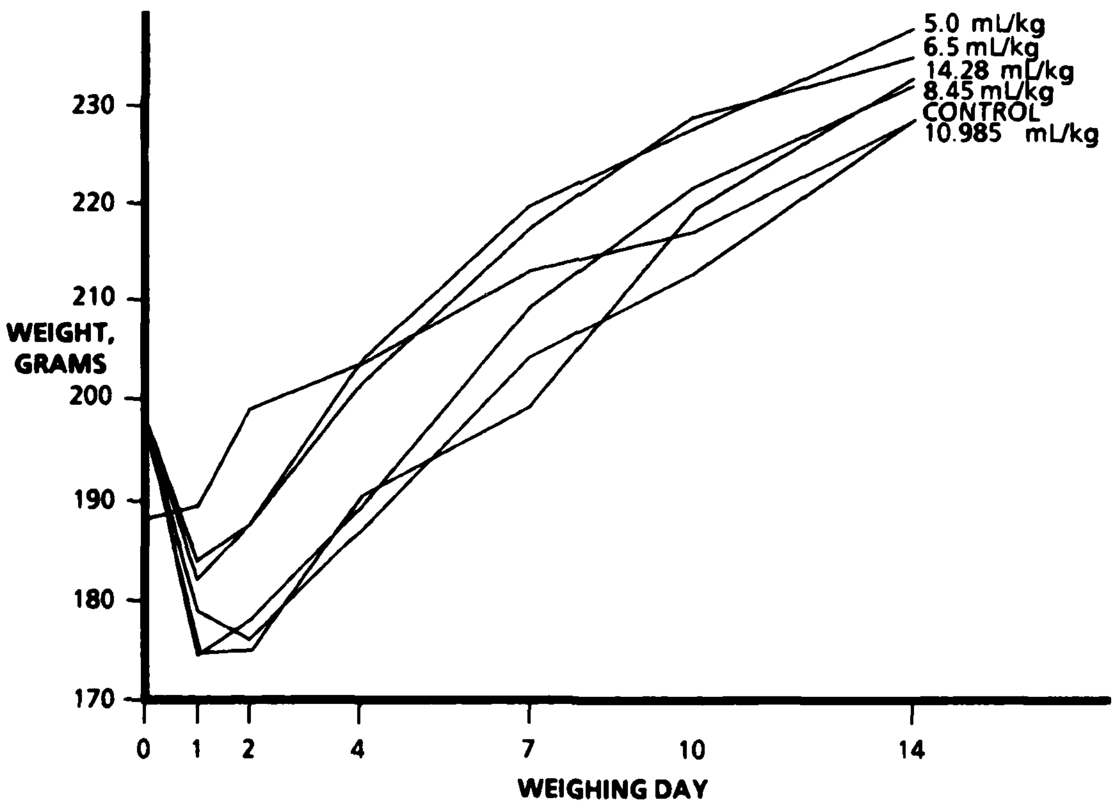

Figure 2. Body weights of female rats given intraperitoneal injections of MIL-H-19457C.

Female rats also lost weight immediately after dosing. However, weight recovery during the 14-day observation period was more complete than that seen with male rats. At the end of 14 days, the mean body weights of all female rats in exposed groups were equal to or greater than corn-oil-treated controls.

Gross necropsy of rats dosed with MIL-H-19457C and MIL-H-19457B revealed a white plaque-like material on the abdominal organs. A similar type material was also present in the control group injected intraperitoneally with corn oil during the MIL-H-19457C LD_{50} tests. Microscopically, the animals dosed with corn oil or dilutions of MIL-H-19457C or MIL-H-19457B in corn oil displayed mild to severe granulomatous perihepatitis and peritonitis. These lesions were attributed to the corn oil vehicle. Secondary liver changes, including hepatocellular swelling and necrosis, were occasionally seen in MIL-H-19457C-dosed rats. These lesions were also thought to be associated with the peritonitis rather than MIL-H-19457C. Fibrotic adhesions of various internal organs were noted in the limited number of rats examined after MIL-H-22072B intraperitoneal injection. In addition, the liver appeared swollen in many cases. A diffuse, mild to moderate, toxic tubular nephrosis, with abundant oxalate crystals, was present in all males dosed intraperitoneally with MIL-H-22072B, whereas distinct renal changes were not observed in females similarly exposed.

ACUTE INHALATION TOXICITY

No deaths occurred in any of the groups of male and female rats exposed to the hydraulic fluid aerosols. In all cases, mild lethargy lasting 1 to 3 hours postexposure was noted in the rats immediately upon removal from the chamber. The fur of animals exposed to MIL-H-19457C and MIL-H-19457B was unkempt and wetted with the hydraulic fluid, while the fur of the animals exposed to MIL-H-22072B appeared normal.

The mass concentrations and particle sizes of aerosol measured in the animal exposures are shown in Tables 7 and 8, respectively.

Two sets of particle size results are presented for MIL-H-22072B. The first set represents sampling over the first 2-hour period. The second set represents sampling over the last 2 hours of exposure. An apparent increase in particle size occurred during the course of the exposure. We believe this was caused by selective vaporization of the relatively volatile aqueous component of MIL-H-22072B (ethylene glycol). Particles that were recycled into the generation pool by impaction on the generation vessel walls and introduction lines had less volatile components than the initial material. As the volatile composition of the MIL-H-22072B generation pool decreased, less particle shrinkage occurred with aging of the aerosol in the exposure chamber.

Body weights of rats exposed via inhalation are shown in Table 9. During the first 24-hour period, little or no weight gain was observed in male or female rats exposed to MIL-H-19457C or MIL-H-19457B. Male or female rats exposed to MIL-H-22072B lost a small amount of weight during this period, but subsequent weighings demonstrated normal growth.

Microscopic examination of the respiratory tissues of control rats and those exposed to the hydraulic fluids indicated mild to moderate, patchy, interstitial pneumonia with associated perivascular cuffs of mononuclear inflammatory cells and occasional aggregates of alveolar macrophages. The changes in the rats exposed to MIL-H-19457C and MIL-H-19457B were equal in severity to the controls, and were therefore thought to be incidental background changes. The respiratory changes were considered to be more severe in the rats exposed to MIL-H-22072B and were compatible with a mild to moderate chemical-induced pneumonitis.

TABLE 7. MASS CONCENTRATION ANALYSIS OF AEROSOL IN ACUTE 4-HOUR AEROSOL INHALATION TESTS

Material	Sex	Concentration (mg/L)[a]
MIL-H-19457C	M	5.79 ± 0.25 (5.20-6.20)
	F	6.31 ± 0.37 (5.74-7.07)
MIL-H-19457B	M	6.19 ± 0.20 (5.84-6.53)
	F	6.35 ± 0.24 (5.63-6.61)
MIL-H-22072B	M	6.84 ± 0.36 (6.10-7.24)
	F	7.56 ± 0.50 (6.55-8.54)

[a]Mean ± SD (range).

TABLE 8. PARTICLE SIZE[a] OF AEROSOLS USED IN ACUTE 4-HOUR AEROSOL INHALATION TESTS

Material	Sex	MMAD (μm)[a]	GSD[b]
MIL-H-19457C	M	2.6	2.1
	F	2.6	2.0
MIL-H-19457B	M	2.5	2.1
	F	2.5	2.0
MIL-H-22072B	M	1.7[c]	1.9
		2.3[d]	2.0
	F	1.7[c]	1.7
		2.3[d]	2.1

[a]Mass median aerodynamic diameter.

[b]Geometric standard deviation.

[c]Initial 2-hour period.

[d]Last 2-hour period.

TABLE 9. BODY WEIGHTS (g)[a] OF RATS EXPOSED VIA INHALATION TO HYDRAULIC FLUID

Day	MIL-H-19457C	MIL-H-19457B	MIL-H-22072B
		Males	
0	214 ± 9	254 ± 6	259 ± 8
1	214 ± 8	257 ± 11	256 ± 7
2	222 ± 10	262 ± 9	259 ± 13
4	239 ± 12	274 ± 8	273 ± 13
7	262 ± 14	290 ± 12	292 ± 11
10	282 ± 17	311 ± 8	309 ± 13
14	304 ± 16	338 ± 9	330 ± 14
		Females	
0	179 ± 4	196 ± 3	190 ± 10
1	179 ± 7	200 ± 2	186 ± 9
2	184 ± 6	203 ± 4[b]	191 ± 9
4	189 ± 8	209 ± 5	191 ± 8
7	200 ± 9	212 ± 4	199 ± 9
10	205 ± 11	213 ± 5	207 ± 9
14	220 ± 13	227 ± 4	210 ± 8

[a]Mean + SD, N = 5 rats/group

[b]Weighed on day 3

ACUTE DERMAL TOXICITY

No signs of toxicity were noted in the rabbits receiving a single dermal application of 5 mL hydraulic fluid/kg body weight. Body weights of the rabbits for the 14-day observation period are shown in Table 10. A slight weight loss was seen shortly after dosing in male rabbits treated with MIL-H-19457B and in male and female rabbits dosed with MIL-H-22072B. Overall weight gain through the 14-day observation period was slightly less in rabbits given MIL-H-19457B when compared to rabbits receiving either MIL-H-19457C or MIL-H-22072B. There was no evidence of skin irritation resulting from dermal contact with any of the three hydraulic fluids, and gross necropsy of the rabbits on day 14 failed to reveal any exposure-related lesions.

TABLE 10. BODY WEIGHTS (kg)[a] OF RABBITS DERMALLY EXPOSED TO HYDRAULIC FLUID

Day	Male	Female
	MIL-H-19457C	
0	2.21 ± 0.26	2.15 ± 0.24
1	2.28 ± 0.24	2.21 ± 0.29
2	2.28 ± 0.23	2.26 ± 0.26
4	2.44 ± 0.25	2.30 ± 0.34
7	2.54 ± 0.25	2.42 ± 0.38
14	2.70 ± 0.22	2.53 ± 0.28
	MIL-H-19457C	
0	2.43 ± 0.27	2.73 ± 0.25
1	2.32 ± 0.32	2.79 ± 0.41
2	2.36 ± 0.33	2.67 ± 0.22
4	2.45 ± 0.33	2.80 ± 0.19
7	2.49 ± 0.29	2.87 ± 0.14
14	2.58 ± 0.29	2.94 ± 0.15
	MIL-H-19457C	
0	2.21 + 0.26	2.15 + 0.24
1	2.28 + 0.24	2.21 + 0.29
2	2.28 + 0.23	2.26 + 0.26
4	2.44 + 0.25	2.30 + 0.34
7	2.54 + 0.25	2.42 + 0.38
14	2.70 + 0.22	2.53 + 0.28

[a]Mean ± SD, N = 5 rabbits/group.

21-DAY REPEATED DOSE INHALATION TOXICITY

A summary of the chemical analysis results obtained during the course of the hydraulic fluid exposures is shown in Table 11. Measured mean concentrations were very close to the desired target values for all exposures. Comparison of the measured concentrations of MIL-H-19457C and of MIL-H-19457B with nominal values indicated a recovery of approximately 65 percent. Examination of particle size information shows that the aerosols of all three hydraulic fluids were respirable.

No deaths occurred upon repeated inhalation exposure to MIL-H-19457C. Transient mild diarrhea was noted in two rabbits in the 25-mg/m^3 exposure and one rabbit in the 250-mg/m^3 exposure. No other overt toxic signs were noted in any of the other species exposed to MIL-H-19457C. Two female rabbits in the low-level MIL-H-19457B exposure died shortly after exposure initiation. Gross necropsy of the rabbits revealed a purulent exudate present in the

TABLE 11. SUMMARY OF 21-DAY EXPOSURE CONCENTRATIONS AND AEROSOL PARTICLE SIZES OF MIL-H-19457C, MIL-H-19457B, MIL-H-22072B

	Target (mg/m^3)	Nominal[a] (mg/m^3)	Measured[b] (mg/m^3)	MMAD[c] (μm)	GSD[d]
MIL-H-19457C	25.00	39 ± 1.0	26.0 ± 0.3	2.3 ± 0.05	1.8 ± 0.05
MIL-H-19457C	250.00	402 ± 15.0	260.0 ± 4.9	2.2 ± 0.05	2.0 ± 0.05
MIL-H-19457B	25.00	39 ± 0.8	26.0 ± 0.3	2.3 ± 0.05	2.2 ± 0.05
MIL-H-19457B	250.00	392 ± 8.3	251.0 ± 3.1	2.3 ± 0.05	2.1 ± 0.05
MIL-H-22072B	25.00				
(Ethylene Glycol)	9.37	e	9.4 ± 0.28	-	-
(Aerosol)	3.75	e	3.8 ± 0.03	1.6 ± 0.05	2.0 ± 0.03
MIL-H-22072B	250.00				
(Ethylene Glycol)	93.70	e	90.0 ± 1.0	-	-
(Aerosol)	37.50	e	38.0 ± 0.3	1.6 ± 0.03	2.0 ± 0.03

[a]Mean + SE, N = 15, calculated; total amount used per day/total airflow per day.

[b]Mean ± SE, N = 15.

[c]Mass median aerodynamic diameter, mean ± SE, N = 13 to 15.

[d]Geometric standard deviation

[e]Not calculated.

thoracic cavity, characteristic of Pasteurella. Bacteriologic cultures of the exudate confirmed the presence of *Pasteurella multocida*. One male hamster died after nine exposures to 250 mg/m^3 MIL-H-19457B. No overt symptoms of toxicity were noted in any of the animals exposed to MIL-H-22072B.

The body weights of animals exposed to the hydraulic fluid aerosols are shown in Tables 12, 13, and 14. Exposure to MIL-H-19457C, MIL-H-19457B, or MIL-H-22072B for 21 days generally did not adversely alter normal growth in any of the species tested.

Results of the hematology and clinical chemistry tests performed on the blood of male and female rats are presented in Tables 15 and 16, respectively. All of the values were within normal biological variation for the species. None of the three hydraulic fluids produced dose-related changes in the blood parameters measured in rats. Similarly, examination of rabbit blood (Tables 17 and 18, male and female, respectively) indicated no biologically significant changes resulting from inhalation of the hydraulic fluid aerosols.

Organ weights measured at the time of necropsy are shown in Tables 19 and 20 for male and female rats, respectively. Heart, spleen, kidney, and brain weights of MIL-H-19457C-exposed rats were statistically equivalent to unexposed controls at $p \leq 0.05$. Increased liver weights of approximately 18 percent were noted in males and females exposed to 250 mg/m^3 MIL-H-19457C when compared to control values, but exposure to 25 mg/m^3 did not produce liver weight changes. A similar effect on the liver was noted in the rats exposed to the other triarylphosphate, MIL-H-19457B. Exposure to the ethylene glycol-based MIL-H-22072B did not result in increased liver weight.

TABLE 12. EFFECT OF REPEATED 6-HOUR INHALATION OF MIL-H-19457C AEROSOL ON ANIMAL BODY WEIGHT[a]

Number of Exposures	Control	25 mg/m^3	250 mg/m^3
	Male Rats (g)		
0	160 ± 1	158 ± 2	156 ± 2
4	172 ± 3	171 ± 3	170 ± 3
6	184 ± 2	172 ± 6	169 ± 4[b]
9	188 ± 2	183 ± 6	174 ± 5[b]
11	187 ± 3	191 ± 5	185 ± 4
14	206 ± 2	201 ± 5	200 ± 5
	Female Rats (g)		
0	119 ± 3	122 ± 3	121 ± 2
4	123 ± 2	127 ± 3	126 ± 2
6	129 ± 2	129 ± 3	126 ± 2
9	131 ± 3	133 ± 3	131 ± 3
11	133 ± 2	136 ± 3	131 ± 2
14	138 ± 3	138 ± 3	137 ± 2
	Male Hamsters (g)		
0	113 ± 5	103 ± 2	100 ± 3[b]
4	108 ± 4	102 ± 2	97 ± 3[b]
6	109 ± 4	104 ± 2	100 ± 3[b]
9	110 ± 4	106 ± 3	101 ± 3
11	110 ± 4	107 ± 3	102 ± 3
14	112 ± 4	109 ± 3	103 ± 3
	Male Rabbits (kg)		
0	4.16 ± 0.18	3.90 ± 0.07	4.53 ± 0.25
4	4.15 ± 0.22	3.93 ± 0.09	4.53 ± 0.22
6	4.22 ± 0.19	4.08 ± 0.09	4.33 ± 0.20
9	4.23 ± 0.21	4.04 ± 0.10	4.55 ± 0.20
11	4.28 ± 0.20	3.95 ± 0.09	4.54 ± 0.19
14	4.24 ± 0.23	3.98 ± 0.09	4.48 ± 0.21
	Female Rabbits (kg)		
0	4.40 ± 0.25	4.50 ± 0.44	4.09 ± 0.25
4	4.42 ± 0.20	4.49 ± 0.47	4.17 ± 0.22
6	4.44 ± 0.22	4.67 ± 0.48	4.07 ± 0.25
9	4.48 ± 0.20	c	4.25 ± 0.18
11	4.41 ± 0.18	4.66 ± 0.45	4.32 ± 0.20
14	4.35 ± 0.15	4.71 ± 0.43	4.30 ± 0.20

[a]Mean ± SE, N = 10 rats or hamsters/group and 4 rabbits/group.

[b]Different from control, $p \leq 0.05$

[c]Not available.

TABLE 13. EFFECT OF REPEATED 6-HOUR INHALATION OF MIL-H-19457B AEROSOL ON ANIMAL BODY WEIGHT

Number of Exposures	Control	25 mg/m^3	250 mg/m^3
Male Rats (g)			
0	174 ± 4	181 ± 17.8^c	189 ± 3^b
4	187 ± 4	197 ± 11.2^c	198 ± 3^b
6	191 ± 4	204 ± 12.1^{b,c}	206 ± 3^b
9	203 ± 4	213 ± 12.2^c	216 ± 3^b
11	207 ± 5	222 ± 12.6^{b,c}	225 ± 4^b
14	219 ± 5	232 ± 13.1^{b,c}	235 ± 4^b
Female Rats (g)			
0	132 ± 2	131 ± 1	136 ± 2
4	135 ± 2	135 ± 1	136 ± 2
6	135 ± 2	137 ± 1	138 ± 2
9	142 ± 3	143 ± 1	142 ± 2
11	143 ± 3	144 ± 1	144 ± 2
14	149 ± 3	150 ± 2	149 ± 2
Male Hamsters (g)			
0	116 ± 2	115 ± 3	113 ± 3
4	111 ± 2	113 ± 3	111 ± 3
6	112 ± 3	114 ± 3	111 ± 3
9	114 ± 3	116 ± 3	112 ± 3
11	115 ± 3	117 ± 3	115 ± 3^c
14	115 ± 3	119 ± 3	115 ± 3^c
Male Rabbits (kg)			
0	4.16 ± 0.18	4.02 ± 0.20	4.30 ± 0.16
4	4.15 ± 0.22	4.14 ± 0.20	4.43 ± 0.14
6	4.22 ± 0.19	4.20 ± 0.19	4.38 ± 0.12
9	4.23 ± 0.21	4.15 ± 0.19	4.35 ± 0.15
11	4.28 ± 0.20	4.21 ± 0.21	4.44 ± 0.13
14	4.24 ± 0.23	4.22 ± 0.22	4.48 ± 0.30
Female Rabbits (kg)			
0	4.40 ± 0.25	4.21 ± 0.44	4.03 ± 0.17
4	4.42 ± 0.20	4.58 ± 0.54^d	3.97 ± 0.24
6	4.44 ± 0.22	4.65 ± 0.47^d	4.01 ± 0.22
9	4.48 ± 0.20	4.75 ± 0.54^d	4.04 ± 0.22
11	4.41 ± 0.18	4.70 ± 0.49^d	4.05 ± 0.20
14	4.35 ± 0.15	4.81 ± 0.53^d	4.10 ± 0.20

[a] Mean ± SE, N = 10 rats or hamsters/group and 4 rabbits/group.
[b] Different from control, $p \leq 0.05$.
[c] N = 9.
[d] M = 2.

TABLE 14. EFFECT OF REPEATED 6-HOUR INHALATION OF MIL-H-22072B AEROSOL ON ANIMAL BODY WEIGHT[a]

Number of Exposures	Control	25 mg/m³	250 mg/m³
		Male Rats (g)	
0	171 ± 1	174 ± 3	172 ± 3
4	182 ± 2	188 ± 3	183 ± 3
6	179 ± 4	186 ± 3	186 ± 3[b]
9	178 ± 4	191 ± 4[b]	187 ± 4[b]
11	188 ± 4	197 ± 4	194 ± 3
14	193 ± 4	204 ± 4[b]	203 ± 3
		Female Rats (g)	
0	125 ± 2	123 ± 1	128 ± 2
4	129 ± 2	130 ± 1	132 ± 2
6	131 ± 2	130 ± 1	133 ± 2
9	131 ± 2	132 ± 2	136 ± 2
11	138 ± 2	132 ± 2	135 ± 3
14	143 ± 3	140 ± 1	141 ± 3
		Male Hamsters (g)	
0	105 ± 3	110 ± 2	112 ± 2
4	103 ± 3	107 ± 3	109 ± 2[b]
6	104 ± 3	110 ± 3	112 ± 2[b]
9	106 ± 3	110 ± 3	111 ± 3
11	109 ± 3	112 ± 3	112 ± 3
14	110 ± 3	114 ± 3	114 ± 3
		Male Rabbits (kg)	
0	4.19 ± 0.11	4.18 ± 0.08	4.52 ± 0.06[b]
4	4.37 ± 0.10	4.40 ± 0.07	4.78 ± 0.06[b]
6	4.29 ± 0.11	4.40 ± 0.08	4.70 ± 0.10[b]
9	4.40 ± 0.11	4.38 ± 0.09	4.76 ± 0.06[b]
11	4.45 ± 0.11	4.46 ± 0.09	4.77 ± 0.05[b]
14	4.43 ± 0.14	4.42 ± 0.12	4.77 ± 0.06
		Female Rabbits (kg)	
0	4.00 ± 0.14	4.17 ± 0.08	4.03 ± 0.22
4	4.15 ± 0.17	4.30 ± 0.13	4.17 ± 0.26
6	4.12 ± 0.16	4.31 ± 0.23	4.11 ± 0.21
9	4.15 ± 0.18	4.27 ± 0.08	4.19 ± 0.24
11	4.21 ± 0.19	4.45 ± 0.11	4.25 ± 0.23
14	4.25 ± 0.22	4.33 ± 0.15	4.27 ± 0.22

[a]Mean ± SE, N = 10 rats or hamsters/group and 4 rabbits/group.
[b]Different from control, $p \leq 0.05$.

TABLE 15. EFFECT OF REPEATED 6-HOUR INHALATION OF HYDRAULIC FLUID AEROSOL ON MALE RAT BLOOD[a]

	Control	25 mg/m^3	250 mg/m^3
		MIL-H-19457C	
RBC (x10^6 cell/mm^3)	7.76 ± 0.10	8.01 ± 0.06[b]	8.01 ± 0.07[b]
WBC (x10^3 cell/mm^3)	5.9 ± 0.4	5.3 ± 0.3	5.8 ± 0.3
HCT (percent)	43.2 ± 0.4	44.3 ± 0.4	44.5 ± 0.5
HGB (g/dL)	15.3 ± 0.2	15.4 ± 0.1	15.6 ± 0.2
MCV (μm^3)	55.7 ± 0.5	55.3 ± 0.3	55.5 ± 0.3
MCH (pg)	19.8 ± 0.4	19.2 ± 0.1	19.5 ± 0.1
MCHC (g/dL)	35.6 ± 0.6	34.7 ± 0.3	35.1 ± 0.2
Total Protein (g/dL)	7.03 ± 0.025	7.08 ± 0.29	8.27 ± 0.30[c]
Albumin (g/dL)	3.98 ± 0.14	3.93 ± 0.16	4.43 ± 0.15
SGOT (IU/L)	59 ± 8	51 ± 8	52 ± 2
Creatinine (mg/dL)	0.65 ± 0.02	0.56 ± 0.03[c]	0.58 ± 0.01[c]
BUN (mg/dL)	17.0 ± 0.8	15.8 ± 0.7	14.9 ± 0.5
Alk. Phos. (IU/L)	166 ± 5	159 ± 3	155 ± 3
Calcium (mg/dL)	11.0 ± 0.4	9.5 ± 0.3[c]	10.9 ± 0.3
		MIL-H-19457B	
RBC (x10^6 cell/mm^3)	7.75 ± 0.10	7.74 ± 0.14	8.43 ± 0.09[b]
WBC (x10^3 cell/mm^3)	4.9 ± 0.2	4.9 ± 0.2	5.43 ± 0.3
HCT (percent)	41.9 ± 0.6	42.5 ± 0.7	45.5 ± 0.5[b]
HGB (g/dL)	15.5 ± 0.1	15.9 ± 0.2	16.2 ± 0.2[b]
MCV (μm^3)	54.1 ± 0.4	54.9 ± 0.3	54.0 ± 0.3
MCH (pg)	20.0 ± 0.2	20.5 ± 0.3	19.3 ± 0.1[c]
MCHC (g/dL)	37.0 ± 0.6	37.4 ± 0.5	35.7 ± 0.2[c]
Total Protein (g/dL)	6.22 ± 0.23	7.43 ± 0.39	7.04 ± 0.39
Albumin (g/dL)	4.38 ± 0.15	4.75 ± 0.11	4.70 ± 0.26
SGOT (IU/L)	50 ± 2	65 ± 13	49 ± 2
Creatinine (mg/dL)	0.57 ± 0.02	0.68 ± 0.03[c]	0.69 ± 0.03[c]
BUN (mg/dL)	13.9 ± 0.6	13.5 ± 0.4	18.1 ± 0.9[b]
Alk. Phos. (IU/L)	124 ± 4	110 ± 4	101 ± 5[b]
Calcium (mg/dL)	10.6 ± 1.1	9.5 ± 0.9	9.0 ± 0.6
		MIL-H-22072B	
RBC (x10^6 cell/mm^3)	8.25 ± 0.11	8.10 ± 0.7	8.70 ± 0.12[b]
WBC (x10^3 cell/mm^3)	5.1 ± 0.1	4.3 ± 0.2[c]	5.8 ± 0.3[c]
HCT (percent)	43.4 ± 0.7	42.8 ± 0.4	46.0 ± 0.8[b]
HGB (g/dL)	14.5 ± 0.2	14.1 ± 0.1	15.2 ± 0.2[c]
MCV (μm^3)	52.6 ± 0.3	52.8 ± 0.2	52.9 ± 0.3
MCH (pg)	17.6 ± 0.1	17.4 ± 0.1[c]	17.4 ± 0.1[c]
MCHC (g/dL)	33.5 ± 0.2	33.0 ± 0.2[c]	32.9 ± 0.1[b]
Total Protein (g/dL)	6.63 ± 0.10	6.90 ± 0.19	7.09 ± 0.28
Albumin (g/dL)	4.63 ± 0.06	4.55 ± 0.01	4.59 ± 0.05
SGOT (IU/L)	66 ± 3	69 ± 3	51 ± 2[b]
Creatinine (mg/dL)	0.69 ± 0.03	0.58 ± 0.03[c]	0.67 ± 0.03
BUN (mg/dL)	16.4 ± 0.7	15.8 ± 0.4	16.7 ± 0.7
Alk. Phos. (IU/L)	119 ± 3	119 ± 3	97 ± 3[b]
Calcium (mg/dL)	10.0 ± 0.2	9.4 ± 0.2	9.0 ± 0.4[c]

[a]Mean ± SE, N = 7 to 10 samples/group
[b]Different from control, $p \leq 0.01$.
[c]Different from control, $p \leq 0.05$.

TABLE 16. EFFECT OF REPEATED 6-HOUR INHALATION OF HYDRAULIC FLUID AEROSOL ON FEMALE RAT BLOOD[a]

	Control	25 mg/m³	250 mg/m³
MIL-H-19457C			
RBC (x10⁶ cell/mm³)	7.42 ± 0.14	7.89 ± 0.08[b]	7.74 ± 0.09[c]
WBC (x10³ cell/mm³)	6.1 ± 0.5	5.3 ± 0.3	5.2 ± 0.4
HCT (percent)	41.3 ± 0.8	44.0 ± 0.5[b]	43.4 ± 0.5[c]
HGB (g/dL)	15.1 ± 0.2	15.4 ± 0.1	15.6 ± 0.2
MCV (µm³)	55.7 ± 0.5	55.8 ± 0.3	56.0 ± 0.5
MCH (pg)	20.4 ± 0.6	19.5 ± 0.2	20.1 ± 0.2
MCHC (g/dL)	36.7 ± 0.9	34.9 ± 0.3	35.9 ± 0.3
Total Protein (g/dL)	8.20 ± 0.16	8.11 ± 0.25	7.92 ± 0.32
Albumin (g/dL)	4.62 ± 0.08	4.58 ± 0.10	4.68 ± 0.11
SGOT (IU/L)	62 ± 3	76 ± 8	48 ± 2[b]
Creatinine (mg/dL)	0.55 ± 0.02	0.59 ± 0.01	0.64 ± 0.03
BUN (mg/dL)	17.8 ± 1.1	16.7 ± 0.7	17.3 ± 1.2
Alk. Phos. (IU/L)	133 ± 2	115 ± 2[b]	109 ± 2[b]
Calcium (mg/dL)	11.0 ± 0.4	11.1 ± 0.7	11.0 ± 0.4
MIL-H-19457B			
RBC (x10⁶ cell/mm³)	7.09 ± 0.13	7.56 ± 0.16[c]	7.57 ± 0.11[b]
WBC (x10³ cell/mm³)	5.1 ± 0.4	4.3 ± 0.3[c]	4.8 ± 0.2
HCT (percent)	38.5 ± 0.7	41.4 ± 1.0[c]	40.9 ± 0.7[c]
HGB (g/dL)	14.9 ± 0.1	15.4 ± 0.2[c]	15.1 ± 0.2
MCV (µm³)	54.2 ± 0.3	54.8 ± 0.3	53.9 ± 0.3
MCH (pg)	21.1 ± 0.4	20.4 ± 0.3	19.9 ± 0.2[c]
MCHC (g/dL)	38.9 ± 0.7	37.2 ± 0.6	37.0 ± 0.5[c]
Total Protein (g/dL)	6.68 ± 0.11	7.17 ± 0.14[c]	7.69 ± 0.26[c]
Albumin (g/dL)	4.69 ± 0.09	4.75 ± 0.05	4.93 ± 0.10
SGOT (IU/L)	70 ± 9	58 ± 2	51 ± 1
Creatinine (mg/dL)	0.66 ± 0.03	0.63 ± 0.03	0.69 ± 0.05
BUN (mg/dL)	18.8 ± 1.4	14.9 ± 0.8	21.3 ± 0.9
Alk. Phos. (IU/L)	86 ± 3	73 ± 8	81 ± 5
Calcium (mg/dL)	10.2 ± 0.6	11.8 ± 2	9.6 ± 1.0
MIL-H-22072B			
RBC (x10⁶ cell/mm³)	7.78 ± 0.14	8.01 ± 0.15	7.95 ± 0.08
WBC (x10³ cell/mm³)	5.2 ± 0.4	5.4 ± 0.3	6.1 ± 0.2[c]
HCT (percent)	41.7 ± 0.7	42.7 ± 0.9	42.1 ± 0.5
HGB (g/dL)	14.7 ± 0.3	15.3 ± 0.3	15.4 ± 0.2
MCV (µm³)	53.6 ± 0.2	53.3 ± 0.2	52.9 ± 0.2[c]
MCH (pg)	18.9 ± 0.2	19.1 ± 0.2	19.4 ± 0.2
MCHC (g/dL)	35.3 ± 0.4	35.9 ± 1.5	36.6 ± 0.5
Total Protein (g/dL)	6.94 ± 0.19	6.95 ± 0.17	7.52 ± 0.18
Albumin (g/dL)	4.67 ± 0.10	5.10 ± 0.18	4.77 ± 0.06
SGOT (IU/L)	74 ± 9	61 ± 3	50 ± 2[b]
Creatinine (mg/dL)	0.77 ± 0.08	0.60 ± 0.03	0.68 ± 0.03
BUN (mg/dL)	26.3 ± 1.9	24.4 ± 1.3	27.2 ± 0.7
Alk. Phos. (IU/L)	97 ± 5	92 ± 4	67 ± 5[b]
Calcium (mg/dL)	10.0 ± 0.1	10.9 ± 0.4	10.2 ± 0.4

[a]Mean ± SE, N = 8 to 10 samples/group.
[b]Different from control, $p \leq 0.01$.
[c]Different from control, $p \leq 0.05$.

TABLE 17. EFFECT OF REPEATED 6-HOUR INHALATION OF HYDRAULIC FLUID AEROSOL ON MALE RABBIT BLOOD[a]

	Control	25 mg/m³	250 mg/m³
MIL-H-19457C			
RBC ($\times 10^6$ cell/mm^3)	5.81 ± 0.07	6.42 ± 0.32	5.70 ± 0.60
WBC ($\times 10^3$ cell/mm^3)	8.8 ± 1.7	8.5 ± 1.4	13.9 ± 1.4
HCT (percent)	37.9 ± 1.1	40.4 ± 1.8	36.5 ± 4.9
HGB (g/dL)	12.8 ± 0.4	13.6 ± 0.5	12.3 ± 1.8
MCV (μm^3)	65.2 ± 1.2	62.9 ± 0.3	63.6 ± 2.4
MCH (pg)	22.1 ± 0.5	21.4 ± 0.2	21.4 ± 1.1
MCHC (g/dL)	33.8 ± 0.2	33.8 ± 0.2	33.6 ± 0.5
Total Protein (g/dL)	6.46 ± 0.70	6.10 ± 0.46	6.64 ± 1.02
Albumin (g/dL)	4.32 ± 0.36	4.36 ± 0.13	4.01 ± 0.67
SGOT (IU/L)	36 ± 4	20 ± 7	21 ± 3
Creatinine (mg/dL)	1.73 ± 0.23	1.43 ± 0.05	1.70 ± 0.15
BUN (mg/dL)	22.4 ± 1.4	14.5 ± 1.6[b]	10.9 ± 1.0[b]
Alk. Phos. (IU/L)	25 ± 7	24 ± 8	33 ± 3
Calcium (mg/dL)	14.8 ± 0.8	14.8 ± 0.3	13.0 ± 1.3
MIL-H-19457B			
RBC ($\times 10^6$ cell/mm^3)	5.81 ± 0.07	6.55 ± 0.09[c]	6.55 ± 0.53[c]
WBC ($\times 10^3$ cell/mm^3)	8.8 ± 1.7	9.3 ± 2.5	11.7 ± 0.8
HCT (percent)	37.9 ± 1.1	41.8 ± 0.6	436 ± 2.6[c]
HGB (g/dL)	12.8 ± 0.4	14.1 ± 0.3	14.9 ± 0.8[c]
MCV (μm^3)	65.2 ± 1.2	63.8 ± 0.4	66.8 ± 2.3
MCH (pg)	22.1 ± 0.5	21.5 ± 0.4	22.8 ± 0.8
MCHC (g/dL)	33.8 ± 0.2	33.8 ± 0.4	34.1 ± 0.4
Total Protein (g/dL)	6.46 ± 0.70	7.51 ± 0.46	6.04 ± 0.19
Albumin (g/dL)	4.32 ± 0.36	5.88 ± 0.26[c]	5.14 ± 0.29
SGOT (IU/L)	36 ± 4	19 ± 4[c]	15 ± 6[c]
Creatinine (mg/dL)	1.73 ± 0.23	1.50 ± 0.15	1.85 ± 0.13
BUN (mg/dL)	22.4 ± 1.4	19.5 ± 1.2	19.6 ± 4.3
Alk. Phos. (IU/L)	25 ± 7	25 ± 3	41 ± 14
Calcium (mg/dL)	14.8 ± 0.8	13.9 ± 1.1	13.7 ± 0.4
MIL-H-22072B			
RBC ($\times 10^6$ cell/mm^3)	5.87 ± 0.33	6.07 ± 0.11	6.45 ± 0.15
WBC ($\times 10^3$ cell/mm^3)	6.7 ± 0.6	9.5 ± 1.1c	8.2 ± 0.7
HCT (percent)	39.5 ± 1.7	39.5 ± 1.2	42.5 ± 1.0
HGB (g/dL)	14.2 ± 0.4	13.7 ± 0.4	14.7 ± 0.3
MCV (μm^3)	67.4 ± 1.6	65.1 ± 1.1	65.8 ± 0.7
MCH (pg)	24.4 ± 1.3	22.6 ± 0.4	22.7 ± 0.6
MCHC (g/dL)	36.1 ± 1.4	34.7 ± 0.2	34.5 ± 0.6
Total Protein (g/dL)	8.75 ± 0.47	9.70 ± 0.27	10.48 ± 0.64
Albumin (g/dL)	5.71 ± 0.17	6.21 ± 0.06	6.44 ± 0.28
SGOT (IU/L)	28 ± 8	26 ± 5	23 ± 3
Creatinine (mg/dL)	1.53 ± 0.13	1.75 ± 0.15	1.83 ± 0.14
BUN (mg/dL)	21.0 ± 1.6	23.6 ± 2.4	29.2 ± 2.3
Alk. Phos. (IU/L)	56 ± 15	47 ± 5	72 ± 27
Calcium (mg/dL)	13.5 ± 0.3	14.9 ± 0.9	14.1 ± 0.6

[a]Mean ± SE, N = 3 to 4 samples/group.
[b]Different from control, $p \leq 0.01$.
[c]Different from control, $p \leq 0.05$

TABLE 18. EFFECT OF REPEATED 6-HOUR INHALATION OF HYDRAULIC FLUID AEROSOL ON FEMALE RABBIT BLOOD[a]

	Control	25 mg/m³	250 mg/m³
		MIL-H-19457C	
RBC (x10⁶ cell/mm³)	6.07 ± 0.38	6.15 ± 0.54	6.11 ± 0.56
WBC (x10³ cell/mm³)	7.4 ± 0.8	7.2 ± 1.2	5.5 ± 0.5
HCT (percent)	37.9 ± 2.9	38.7 ± 2.4	38.6 ± 3.9
HGB (g/dL)	12.9 ± 1.0	13.1 ± 0.8	12.9 ± 1.2
MCV (μm³)	62.3 ± 1.9	63.2 ± 1.6	63.0 ± 0.6
MCH (pg)	21.3 ± 0.7	21.4 ± 0.8	21.3 ± 0.1
MCHC (g/dL)	34.2 ± 0.2	33.9 ± 0.5	33.7 ± 0.3
Total Protein (g/dL)	4.53 ± 0.37	7.00 ± 0.65	6.15 ± 1.27
Albumin (g/dL)	3.15 ± 0.29	4.79 ± 0.41	4.95 ± 1.33
SGOT (IU/L)	22 ± 2	20 ± 7	16 ± 5
Creatinine (mg/dL)	1.98 ± 0.18	1.68 ± 0.37	2.08 ± 0.16
BUN (mg/dL)	17.7 ± 1.3	20.1 ± 6.3	21.8 ± 2.5
Alk. Phos. (IU/L)	27 ± 4	20 ± 3	36 ± 7
Calcium (mg/dL)	13.9 ± 1.3	14.0 ± 0.6	15.5 ± 0.7
		MIL-H-19457B	
RBC (x10⁶ cell/mm³)	6.07 ± 0.38	6.20 ± 0.29	6.99 ± 0.13
WBC (x10³ cell/mm³)	7.4 ± 0.8	6.5 ± 1.3	6.9 ± 1.1
HCT (percent)	37.9 ± 2.9	39.2 ± 0.3	44.1 ± 0.5
HGB (g/dL)	12.9 ± 1.0	13.5 ± 0.3	15.0 ± 0.2
MCV (μm³)	62.3 ± 1.9	63.3 ± 2.5	63.1 ± 0.8
MCH (pg)	21.3 ± 0.7	21.7 ± 0.6	21.4 ± 0.3
MCHC (g/dL)	34.2 ± 0.2	34.3 ± 0.4	33.9 ± 0.06
Total Protein (g/dL)	4.53 ± 0.37	6.56 ± 2.31	7.00 ± 0.65
Albumin (g/dL)	3.15 ± 0.29	5.32 ± 1.55	6.23 ± 0.56[b]
SGOT (IU/L)	22 ± 2	7 ± 3[c]	13 ± 2[b]
Creatinine (mg/dL)	1.98 ± 0.18	1.90 ± 0.30	2.63 ± 0.31
BUN (mg/dL)	17.7 ± 1.3	21.4 ± 2.7	23.0 ± 1.1
Alk. Phos. (IU/L)	27 ± 4	22 ± 8	26 ± 6
Calcium (mg/dL)	13.9 ± 1.3	18.9 ± 2.8	15.9 ± 0.7
		MIL-H-22072B	
RBC (x10⁶ cell/mm³)	5.58 ± 0.36	6.05 ± 0.18	6.04 ± 0.20
WBC (x10³ cell/mm³)	7.0 ± 0.9	7.2 ± 0.9	8.2 ± 0.9
HCT (percent)	36.9 ± 2.2	39.7 ± 1.3	39.1 ± 1.2
HGB (g/dL)	13.7 ± 0.5	14.4 ± 0.4	14.0 ± 0.4
MCV (μm³)	66.1 ± 0.3	65.6 ± 0.4	64.8 ± 0.3[b]
MCH (pg)	24.7 ± 1.0	23.8 ± 0.2	23.2 ± 0.2
MCHC (g/dL)	37.3 ± 1.4	36.3 ± 0.2	35.9 ± 0.2
Total Protein (g/dL)	9.38 ± 0.29	8.98 ± 0.38	9.38 ± 6.34
Albumin (g/dL)	6.08 ± 0.13	5.84 ± 0.17	5.69 ± 0.19
SGOT (IU/L)	36 ± 6	27 ± 4	20 ± 2
Creatinine (mg/dL)	1.75 ± 0.23	1.53 ± 0.14	1.80 ± 0.13
BUN (mg/dL)	28.1 ± 1.6	25.1 ± 3.2	26.1 ± 6.6
Alk. Phos. (IU/L)	49 ± 2	52 ± 7	54 ± 13
Calcium (mg/dL)	14.3 ± 0.2	1.8 ± 0.2	13.0 ± 0.7

[a]Mean ± SE, N = 3 to 4 samples/group.
[b]Different from control, $p \leq 0.05$.
[c]Different from control, $p \leq 0.01$.

TABLE 19. EFFECT OF REPEATED 6-HOUR INHALATION OF HYDRAULIC FLUID AEROSOL ON MALE RAT ORGAN WEIGHT[a]

	Control	25 mg/m³	250 mg/m³
MIL-H-19457C			
Body Wt, g	189 ± 4	198 ± 4	194 ± 3
Heart Wt, g	0.65 ± 0.02	0.67 ± 0.02	0.65 ± 0.02
Heart/100 g Body Wt	0.35 ± 0.01	0.34 ± 0.01	0.33 ± 0.01
Liver Wt, g	6.56 ± 0.19	6.58 ± 0.18	6.39 ± 0.14
Liver/100 g Body Wt	3.47 ± 0.08	3.32 ± 00.04	3.29 ± 0.03
Spleen Wt, g	0.43 ± 0.01	0.49 ± 0.01[b]	0.49 ± 0.01[b]
Spleen/100 g Body Wt	0.23 ± 0.003	0.25 ± 0.003[b]	0.25 ± 0.003[b]
Kidney Wt, g	1.43 ± 0.03	1.47 ± 0.03	1.42 ± 0.03
Kidney/100 g Body Wt	0.76 ± 0.01	0.74 ± 0.01	0.73 ± 0.01
Brain Wt, g	1.69 ± 0.03	1.72 ± 0.02	1.68 ± 0.02
Brain/100 g Body Wt	0.89 ± 0.01	0.87 ± 0.02	0.87 ± 0.01
MIL-H-19457B			
Body Wt, g	216 ± 5	266 ± 4	232 ± 4[b]
Heart Wt, g	0.72 ± 0.03	0.76 ± 0.02	0.77 ± 0.02
Heart/100 g Body Wt	0.33 ± 0.01	0.34 ± 0.01	0.33 ± 0.01
Liver Wt, g	7.08 ± 0.21	7.61 ± 0.18	8.65 ± 0.17[c]
Liver/100 g Body Wt	3.27 ± 0.05	3.37 ± 0.04	3.73 ± 0.04[c]
Spleen Wt, g	0.48 ± 0.01	0.48 ± 0.01	0.49 ± 0.02
Spleen/100 g Body Wt	0.22 ± 0.003	0.21 ± 0.01	0.21 ± 0.01
Kidney Wt, g	1.62 ± 0.04	1.71 ± 0.04	1.75 ± 0.03[b]
Kidney/100 g Body Wt	0.75 ± 0.01	0.76 ± 0.01	0.76 ± 0.01
Brain Wt, g	1.76 ± 0.04	1.75 ± 0.02	1.77 ± 0.01
Brain/100 g Body Wt	0.81 ± 0.01	0.78 ± 0.01[b]	0.76 ± 0.01[c]
MIL-H-22072B			
Body Wt, g	202 ± 3	199 ± 4	200 ± 5
Heart Wt, g	0.71 ± 0.01	0.79 ± 0.06	0.69 ± 0.03
Heart/100 g Body Wt	0.35 ± 0.01	0.40 ± 0.03	0.34 ± 0.01
Liver Wt, g	7.16 ± 0.26	6.81 ± 0.17	8.38 ± 0.26[b]
Liver/100 g Body Wt	3.55 ± 0.09	3.43 ± 0.04	4.18 ± 0.05[b]
Spleen Wt, g	0.46 ± 0.02	0.47 ± 0.01	0.47 ± 0.01
Spleen/100 g Body Wt	0.23 ± 0.01	0.24 ± 0.01	0.23 ± 0.003
Kidney Wt, g	1.64 ± 0.03	1.57 ± 0.04	1.58 ± 0.05
Kidney/100 g Body Wt	0.81 ± 0.01	0.79 ± 0.01	0.79 ± 0.01
Brain Wt, g	1.74 ± 0.02	1.71 ± 0.02	1.71 ± 0.01
Brain/100 g Body Wt	0.86 ± 0.01	0.86 ± 0.02	0.86 ± 0.02

[a]Mean ± SE, N = 9 to 10 samples/group
[b]Different from control, $p \leq 0.01$
[c]Different from control, $p \leq 0.05$

TABLE 20. EFFECT OF REPEATED 6-HOUR INHALATION OF HYDRAULIC FLUID AEROSOL ON FEMALE RAT BODY WEIGHT[a]

	Control	25 mg/m³	250 mg/m³
	MIL-H-19457C		
Fasted Body Wt, g	132 ± 3	133 ± 3	133 ± 2
Heart Wt, g	0.51 ± 0.01	0.54 ± 0.01	0.52 ± 0.01
Heart/100 g Body Wt	0.39 ± 0.01	0.40 ± 0.01	0.39 ± 0.01
Liver Wt, g	4.17 ± 0.11	4.14 ± 0.07	4.97 ± 0.12[b]
Liver/100 g Body Wt	3.16 ± 0.05	3.12 ± 0.06	3.74 ± 0.09[b]
Spleen Wt, g	0.35 ± 0.01	0.36 ± 0.01	0.35 ± 0.01
Spleen/100 g Body Wt	0.26 ± 0.01	0.27 ± 0.01	0.26 ± 0.01
Kidney Wt, g	1.11 ± 0.03	1.11 ± 0.03	1.12 ± 0.02
Kidney/100 g Body Wt	0.84 ± 0.01	0.83 ± 0.02	0.84 ± 0.01
Brain Wt, g	1.58 ± 0.02	1.63 ± 0.03	1.60 ± 0.02
Brain/100 g Body Wt	1.20 ± 0.02	1.22 ± 0.03	1.21 0.01
	MIL-H-19457B		
Fasted Body Wt, g	144 ± 3	142 ± 1	146 ± 2
Heart Wt, g	0.53 ± 0.01	0.52 ± 0.01	0.51 ± 0.01
Heart/100 g Body Wt	0.36 ± 0.01	0.37 ± 0.01	0.35 ± 0.01
Liver Wt, g	4.39 ± 0.14	4.29 ± 0.09	5.03 ± 0.12[c]
Liver/100 g Body Wt	3.04 ± 0.06	3.02 ± 0.04	3.45 ± 0.06[c]
Spleen Wt, g	0.38 ± 0.01	0.37 ± 0.01	0.39 ± 0.01
Spleen/100 g Body Wt	0.26 ± 0.003	0.26 ± 0.01	0.26 ± 0.01
Kidney Wt, g	1.19 ± 0.03	1.09 ± 0.02[b]	1.21 ± 0.03
Kidney/100 g Body Wt	0.82 ± 0.01	0.77 ± 0.02[b]	0.83 ± 0.01
Brain Wt, g	1.64 ± 0.01	1.65 ± 0.02	1.66 ± 0.01
Brain/100 g Body Wt	1.14 ± 0.03	1.16 ± 0.22	1.14 ± 0.02
	MIL-H-22072B		
Fasted Body Wt, g	135 ± 3	133 ± 1	137 ± 3
Heart Wt, g	0.50 ± 0.01	0.48 ± 0.01	0.49 ± 0.01
Heart/100 g Body Wt	0.37 ± 0.01	0.36 ± 0.01	0.36 ± 0.01
Liver Wt, g	4.03 ± 0.10	3.89 ± 0.05	3.93 ± 0.11
Liver/100 g Body Wt	2.99 ± 0.05	2.92 ± 0.03	2.87 ± 0.04
Spleen Wt, g	0.38 ± 0.01	0.36 ± 0.01	0.37 ± 0.01
Spleen/100 g Body Wt	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.003
Kidney Wt, g	1.04 ± 0.02	1.02 ± 0.02	1.03 ± 0.03
Kidney/100 g Body Wt	0.78 ± 0.01	0.76 ± 0.02	0.75 ± 0.01
Brain Wt, g	1.62 ± 0.02	1.58 ± 0.03	1.64 ± 0.02
Brain/100 g Body Wt	1.20 ± 0.02	1.19 ± 0.02	1.20 ± 0.02

[a]Mean + Se, N = 9 to 10 samples/group.
[b]Different from control, $p \leq 0.01$.
[c]Different from control, $p \leq 0.05$.

Microscopic examination of the tissue collected from the animals exposed to hydraulic fluid aerosols did not reveal any significant exposure-related lesions in any of the three species. The majority of the rabbits (control and exposed) in all three studies demonstrated minimal to mild respiratory changes compatible with an undetermined subclinical infection. Minimal to mild inflammatory lung changes and kidney hyaline droplets were noted with equal frequency in the rats exposed to MIL-H-19457C and MIL-H-19457B and their respective controls. Despite the increased liver weight in the rats exposed to MIL-H-19457C or MIL-H-19457B, no significant liver lesions were noted. No significant tissue changes were noted in any of the hamster groups.

NEUROTOXICITY

The resultant mean scores of three observers, 21 days after the initial peroral dose, are compiled in Tables 21 through 24. The vehicle control (corn oil) resulted in all negative scores. Positive neurotoxic symptoms were observed in all chickens that received 75 or 90 mg/kg of TOCP. Three of the four chickens that received 60 mg/kg TOCP showed minor symptoms, while the fourth demonstrated advanced paralytic symptoms at 21 days and ultimately died at 22 days.

One observer recorded minor symptoms for one chicken dosed at the highest level of MIL-H-19457C; otherwise, all scoring of the MIL-H-19457C-dosed chickens was negative. Significant neurotoxic symptoms were seen in chickens at all but the lowest dose level of MIL-H-19457B. The 300- and 420-mg/kg dose levels each had one chicken dead at 21 days. At that time, all surviving chickens at the three high dose levels demonstrated positive to advanced neurotoxic symptoms. Final mortality of the MIL-H-19457B-treated hens, 30 days following the initial peroral dose, was 3/4 at the 420-mg/kg level and 2/4 at the 360- and 300-mg/kg levels. No deaths occurred at the 240-mg/kg level.

The groups chosen for calculation of TOCP equivalent were 300 mg/kg MIL-H-19457B and 75 mg/kg TOCP. These were the lowest concentration groups showing significant neurotoxic signs. Using the standard formula, MIL-H-19457B has a TOCP equivalent of 28.4 percent. Since no effects were noted with MIL-H-19457C, it has a TOCP equivalent of zero percent.

No dose-related histopathologic changes were seen in the neuropil of rats treated with MIL-H-19457C or corn-oil-treated rats. The only degenerative changes noted in these groups consisted of complete loss of randomly scattered axons, which was believed to be related to postmortem autolysis.

Microscopic changes observed in the MIL-H-19457B-treated hens were identical to the changes seen in the TOCP-dosed hens, although the severity of the changes in the TOCP-dosed hens was greater. Axonal degeneration and demyelinization were noted in both groups and appeared to be sequential changes. The sequence probably began with swelling of the axon, resulting in myelin compression and ellipsoid formation. These events were followed by axonal fragmentation and lysis and, finally, by the complete loss of myelin. The lesions considered to be related to MIL-H-19457B exposure were degeneration and demyelinization in the white matter of the spinal cord.

TABLE 21. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF CORN OIL

Animal Number	Dose (mg/kg)	21-Day Mean Observation Score	Total Score Per Group
18219	1	0	
18224	1	0	
18227	1	0	
18232	1	0	
18235	1	0	
18240	1	0	0
18201	1	0	
18213	1	0	
18241	1	0	
18256	1	0	
18257	1	0	
18259	1	0	

TABLE 22. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF TRIORTHOCRESYLPHOSPHATE (TOCP)

Animal Number	Dose (mg/kg)	21-Day Mean Observation Score	Total Score Per Group
18208	60	4	
18215	60	2	22
18217	60	4	
18238	60	12	
18239	75	8	
18250	75	10.7	38.7
18209	75	12	
18210	75	8	
18216	90	12	
18223	90	10.7	38.7
18242	90	8	
19258	90	8	

TABLE 23. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF MIL-H-19457C

Animal Number	Dose (mg/kg)	21-Day Mean Observation Score	Total Score Per Group
18202	240	0	
18204	240	0	0
18211	240	0	
18212	240	0	
18214	300	0	
18230	300	0	0
18243	300	0	
18260	300	0	
19218	360	0	
18220	360	0	0
18222	360	0	
18225	360	0	
18226	420	0	
18244	420	0	0.7
18247	420	0.7	
18248	420	0	

TABLE 24. SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF MIL-H-19457B

Animal Number	Dose (mg/kg)	21-Day Mean Observation Score	Total Score Per Group
18203	240	0	
18205	240	4	12
18228	240	0	
18229	240	8	
18231	300	8	
18233	300	8	44
18246	300	16	
18252	300	12	
19206	360	8	
18207	360	8	34
18221	360	12	
18236	360	6	
18251	420	12	
18253	420	8	44
18254	420	8	
18255	420	16	

DISCUSSION

No eye or skin irritation resulted from administration of any of these three hydraulic fluids. All three hydraulic fluids tested were not irritants. In addition, none of the materials demonstrated any sensitization potential.

Single dose oral exposure to the two triarylphosphate fluids produced mild diarrhea with concomitant weight loss. However, these effects quickly disappeared and probably were related to the laxative effect of the corn oil. The absence of mortality in any of the groups receiving oral doses of the hydraulic fluids at 5 mL/kg indicates a very low order of oral toxicity.

Intraperitoneal injection of the hydraulic fluids generally resulted in more severe signs of toxicity compared to oral exposure. However, even at the large dose of 5 mL/kg, mortality was absent in rats given MIL-H-19457B or MIL-H-22072B. The LD_{50} for MIL-H-19457C was determined to be in excess of two times the initial screening dose for both male and female rats. These data further indicate a low order of toxicity.

Exposure through 4-hour aerosol inhalation or 24 hours of acute dermal contact also failed to produce mortality.

The most significant effect noted in rats, rabbits, or hamsters repeatedly exposed for 6 hours each day to the two triarylphosphate hydraulic fluids was increased liver weight. This effect was seen in the rats exposed at 250 mg/m^3 but was absent in the rats exposed at 25 mg/m^3. The liver weight change was not accompanied by any concomitant increases in the measured blood serum liver function indices. In addition, the microscopic examination of the liver tissue obtained from the rats failed to establish any exposure-related lesions. The increased liver weight was thought to be a compensatory response to the increased metabolic needs of the liver, probably through proliferation of the endoplasmic reticulum. Ultrastructural examination of the liver was not conducted in this study, but could be useful, along with an examination of the liver microsomal enzyme system, in confirming this hypothesis.

The glycol-based fluid, MIL-H-22072B, generally failed to demonstrate any significant toxic signs after repeated inhalation exposure. Ethylene glycol, accounting for about 38 percent of the MIL-H-22072B fluid, has been reported to produce oxalate crystals in the tubules of the kidney (Bove, 1966). Oxalate crystals were not evident in the kidneys of any of the three species intermittently exposed via inhalation to an estimated ethylene glycol concentration of 94 mg/m^3 at the 250-mg/m^3 MIL-H-22072B exposure level. Oxalate crystals were, however, observed in the kidneys of male rats receiving MIL-H-22072B intraperitoneally at a dose of 5 mL/kg. Coon et al. (1970) reported that rabbits developed moderate to severe eye irritation, and rats developed corneal opacity after 8 days of continuous exposure to ethylene glycol at a concentration of 12 mg/m^3. Although eye tissues rom the animals exposed to MIL-H-22072B were not examined microscopically, there were no gross indications of corneal opacity or eye irritation.

Many organophosphorus compounds, including TOCP, have been found to cause delayed neurotoxic effects in man (Doull et al., 1979). A single exposure to a neurotoxic organophosphorus compound has been reported to be capable of producing axonal damage after a delay of 8 to 10 days. Low-level nerve injury may occur in humans after chronic exposure to these compounds. Similar neurotoxic effects have been demonstrated in adult chickens and cats after exposure to TOCP (Beresford and Glees, 1963). Siegel et al. (1965) demonstrated the neurotoxic effect of a triarylphosphate hydraulic fluid previously thought to be nontoxic in animals. Johannsen et al. (1977) demonstrated that phosphates prepared from ortho alkyl substituted phenols are often neurotoxic if the ortho alkyl group has at least one hydrogen atom on the α carbon for activation.

They also demonstrated that increased substitution or branching of that group resulted in decreased neurotoxicity. These findings were also demonstrated by Bondy et al. (1960).

The neurotoxic screening test used in the present study demonstrated that MIL-H-19457B was a neurotoxic agent with significant axonal damage developing after exposure. MIL-H-19457C, on the other hand, was not found to be neurotoxic. Chemical analysis of the MIL-H-19457B sample conducted by Vernot et al. (1982) indicated an o-cresol content less than 0.2 percent. GC/MS analysis of MIL-H-19457B indicated the presence of a sufficient concentration of phenols associated with neurotoxicity as phosphates to account for the neurotoxic effects of MIL-H-19457B. This led to the suggestion that the determination of the overall concentration of neurotoxic phosphates in the hydraulic fluids may be a more reliable indicator of neurotoxic potential than total o-cresol content. Analysis of MIL-H-19457C indicated total o-cresol content of less than 0.02 percent. GC/MS analysis of MIL-H-19457C showed 87 percent as either phenol or para-tertiary butylphenol. Only a small fraction of MIL-H-19457C consisted of *ortho* alkyl substituted phenols. Apparently, this structural constitution reduced the neurotoxic potential because of the reduced presence of neurotoxic esters.

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Acute toxicity studies including eye and skin irritation, skin sensitization, single dose oral, single dose intraperitoneal, 4-hour aerosol inhalation, single dose dermal, and 21-day repeated aerosol inhalation were conducted with the hydraulic fluids MIL-H-19457C, MIL-H-19457B and MIL-H-22072B. The first two hydraulic fluids are triarylphosphate ester-based materials, while the third is glycol based. MIL-H-19457C and MIL-H-19457B were also tested for neurotoxic potential with a repeated dose screening assay in chickens. None of the three hydraulic fluids produced eye or skin irritation in rabbits. Guinea pig sensitization tests were also negative for all three fluids. Single oral doses of 5 mL/kg of any of the materials failed to produce mortality in male or female Sprague-Dawley rats. Diarrhea and weight loss were seen in the rats given either MIL-H-19457C or MIL-H-19457B. These effects were not seen in the rats dosed with MIL-H-22072B. Single dose intraperitoneal LD_{50} values were also determined.

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